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Short Communication

Chromosomal localization of microsatellite loci in *Drosophila mediopunctata*

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Abstract

Drosophila mediopunctata has been used as a model organism for genetics and evolutionary studies in the last three decades. A linkage map with 48 microsatellite loci recently published for this species showed five syntenic groups, which had their homology determined to *Drosophila melanogaster* chromosomes. Then, by inference, each of the groups was associated with one of the five major chromosomes of *D. mediopunctata*. Our objective was to carry out a genetic (chromosomal) analysis to increase the number of available loci with known chromosomal location. We made a simultaneous analysis of visible mutant phenotypes and microsatellite genotypes in a backcross of a standard strain and a mutant strain, which had each major autosome marked. Hence, we could establish the chromosomal location of seventeen loci; including one from each of the five major linkage groups previously published, and twelve new loci. Our results were congruent with the previous location and they open new possibilities to future work integrating microsatellites, chromosomal inversions, and genetic determinants of physiological and morphological variation.

Keywords: *tripunctata*, SSR, genetic chromosomal analysis, BLAST search, Muller's elements.

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Chromosomal localization of genetic markers is an essential tool for the identification of factors associated to biological traits (Carvalho and Klaczko, 1993; Cazemajor *et al.*, 1997; Noor *et al.*, 2000; Hatadani *et al.*, 2004). Several traits are mapped using microsatellite loci, for example: growth rates (Laine *et al.*, 2013; Ori *et al.*, 2014), behaviour (Oxley *et al.*, 2010; Laine *et al.*, 2014), courtship song (Schäfer *et al.*, 2010; Lagisz *et al.*, 2012), viability (Plough, 2012), male sterility (Moriguchi *et al.*, 2014), pathogen resistance (Stephens *et al.*, 2014), among others. Furthermore, physical and linkage maps may allow insights about evolutionary events that occurred in natural populations and genomic rearrangement within a specific line (Barker *et al.*, 2009; Santos *et al.*, 2010; Simões *et al.*, 2012).

Drosophila mediopunctata is a forest dwelling Neotropical species that has the typical "primitive" *Drosophila* karyotype with five pairs of rods and a pair of dot chromosomes (Kastritsis, 1966). In the last three decades, this species has been used as a model organism for genetics and evolutionary studies (Klaczko, 2006) that characterized chromosomal inversion polymorphism (Peixoto and Klaczko, 1991; Ananina *et al.*, 2004; Batista *et al.*, 2012), morphological variation (Klaczko and Bitner-Mathé, 1990; Andrade *et al.*, 2005) and their association (Bitner-Mathé *et al.*, 1995; Hatadani *et al.*, 2004; Andrade *et al.*, 2009).

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A total of 134 microsatellite loci markers were described for *D. mediopunctata* (Laborda *et al.*, 2009). A linkage map with five syntenic groups for 48 loci was published (Laborda *et al.*, 2012). Each of these groups was associated to one of the five major *D. mediopunctata* chromosomes by cross-reference to BLAST searches on the *D. melanogaster* genome (a 49th locus was assigned to the dot chromosome). The synteny between *D. mediopunctata* and *D. melanogaster* chromosomes was established using fluorescence *in situ* hybridization (*FISH*) in polytene chromosomes of highly conserved genes (Brianti *et al.*, 2013). An analysis of the *Drosophila* 12 Genomes Consortium (2007) data showed that the chromosomes in this genus are highly conserved (Bhutkar *et al.*, 2008); 95% of about 13,000 *D. melanogaster* genes are located on the same Muller element across the twelve species. Thus, the use of BLAST searches to locate a marker is reliable, but a small fraction of markers remains without a completely certain location. Usually, a threshold value for the BLAST e-value in the range of 10^{-3} to 10^{-5} is frequently used to assign homology; thus 10^{-5} represents a conservative approach for multiple comparisons (Domazet-Loso and Tautz, 2003). Therefore, our main objective is to increase the number of available loci assigned to chromosomes.

We performed a chromosomal localization of *D. mediopunctata* microsatellite loci using a genetic (chro-

mosomal) analysis. Three inbred strains were used. Strain CR27A carries mutations with visible effects marking each of the major autosomes: it is heterozygous for the dominant visible and recessive lethal mutation *Delta-5* (Δ-5), located on chromosome II; and it is homozygous for recessive mutations *cabernet* (*cb*), *coral* (*cr*) and *alfinete* (*al*), located on chromosomes III, IV and V, respectively. CR27B shares with CR27A the same genetic background, except for chromosome II, where it is homozygous for the wild type chromosome of CR27A. The other strain (ITA24P) has no marker mutations (wild type alleles for the mutations mentioned above). A description of the strains and the crossing outline are shown in detail by Carvalho and Klaczko (1993) and Hatadani *et al.* (2004).

Briefly, we crossed a CR27A male with an ITA24P virgin female in vials with trimeveledon culture medium (Rocha *et al.*, 2009) at 18 °C. Every other day, the couple was transferred to a new vial for up to six transfers. The analysis of the first generation flies (three F1 males and three F1 females) allowed the identification of sex-linked microsatellite loci by the inheritance pattern (heterozygous females and hemizygous males for X-linked loci). Autosomal microsatellite loci were located analyzing the backcross offspring of an F1 male with a CR27B virgin female. Since there is no recombination in males of *D. mediopunctata* (Cavasini *et al.*, 2010), the microsatellite alleles of CR27A strain always segregate with their syntenic visible mutation (microsatellite locus and visible mutation on the same chromosome). A sample of 48 backcross offsprings was used for this analysis, three individuals from each of the 16 possible phenotypes - permutations of four (mutation or wild) phenotypes. Figure 1 shows an example of this procedure.

The genomic DNA extraction followed the protocol described by Aljanabi and Martinez (1997) with modifications. Amplification of microsatellite loci followed the parameters described in Cavasini *et al.* (2013). Polymerase Chain Reaction (PCR) products were all firstly visualized in 1% agarose gels stained with SYBR® Safe DNA gel staining (Invitrogen); thereafter, if necessary, they were also revealed in 6% polyacrylamide gels stained with $AgNO_3$. Silver staining procedures followed the method described by Creste *et al.* (2001) with modifications.

A total of 36 loci were tested, 17 of which were located (Table 1). It was not possible to locate the other loci due to the presence of shared alleles by the strains used in the crosses. Five loci were used as controls since they were already listed in the linkage map previously published by Laborda *et al.* (2012). Each one is located in one of the five corresponding syntenic groups established by the linkage map, and respectively on the five major *D. mediopunctata* chromosomes. Therefore, our results were congruent with the previous localization established by homology, thus, confirming the location of 49 loci tested by Laborda *et al.* (2012). In addition, 12 loci were added, totaling 61

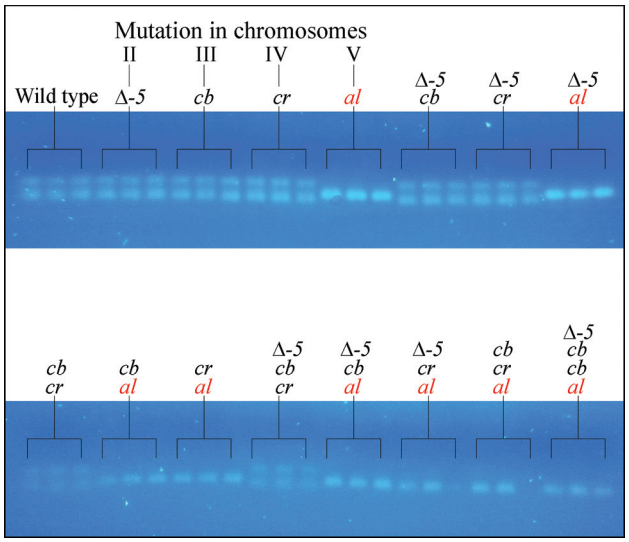

Figure 1 - Sample of 48 backcross offsprings genotyped for Dmed^{UNICAMP}_ssr011 locus (three individuals per phenotype). Mutations *Delta-5* (Δ-5), *cabernet* (*cb*), *coral* (*cr*) and *alfinete* (*al*), are located on chromosomes II, III, IV and V, respectively. All the backcross offsprings that express the recessive mutation *alfinete* (in red) are homozygous for all loci in chromosome V, such as the Dmed^{UNICAMP}-_ssr011 locus. On the other hand, all the backcross offsprings that do not express *alfinete* are heterozygous for all loci in chromosome V, with different alleles in the parental strains.

microsatellite loci with their chromosomal location confirmed. Since CR27A carries no visible marker on the dot chromosome (VI), there is a very low probability (3.6 x 10^{-15}) of mapping any of 15 autosomal loci on dot chromosome (for a similar procedure see Noor *et al.*, 2000).

Table 1 shows BLAST results (E-values) of *D. mediopunctata* sequences matched to the *D. melanogaster* genome. The first BLAST column shows E-values matched to the respective *D. melanogaster* homologous chromosome. The second BLAST column shows the smallest E-value matched to a non-homologous *D. melanogaster* chromosome. Thirteen loci showed E-values consistent to their actual chromosomal location. Nevertheless, the loci Dmed^{UNICAMP}_ssr084 and Dmed^{UNICAMP}_ssr011 (abbreviated Dmed084 and Dmed011, respectively, and similarly henceforward) showed inconclusive BLAST results, with the same E-value for different chromosomes. Nonetheless, our analysis located them unambiguously on chromosomes III and V, respectively. For the Dmed094 and Dmed131 loci, the BLAST results (0.177 and 6.7 x 10^{-5}) point out, respectively, non-significantly to chromosome V and misleadingly to the X chromosome; yet, in fact, both loci are on chromosome IV. Thus, although the gene content in Muller's elements is highly conserved in *Drosophila*, some small sequences may not be easily assigned to homologous chromosomes in other species. In these cases, the genetic (chromosomal) analysis is a powerful tool to elucidate their locations.

Table 1 - Chromosomal localization of 17 microsatellites loci of *D. mediopunctata* by synteny with visible phenotype of mutations (autosomes) or by sex-linked inheritance pattern (sex chromosome).

CHR	Mutation	Locus	BLAST [b]	BLAST [c]
X		Dmed021	9.4×10^{-23}	0.073
		Dmed113 [a]	6.1×10^{-10}	2.142
II	*Delta-5* (Δ-*5*)	Dmed018	0.005	0.019
		Dmed060	1.0×10^{-13}	0.342
		Dmed118 [a]	1.2×10^{-18}	0.059
III	*cabernet* (*cb*)	Dmed067	2.5×10^{-30}	0.427
		Dmed084	1.151	1.151
		Dmed085	4.9×10^{-22}	1.487
		Dmed095	2.2×10^{-16}	0.011
		Dmed106 [a]	0.008	0.129
IV	*coral* (*cr*)	Dmed094	2.761	0.177
		Dmed102 [a]	1.5×10^{-21}	0.071
		Dmed103	2.3×10^{-17}	0.005
		Dmed131	0.064	6.7×10^{-5}
V	*alfinete* (*al*)	Dmed011 [a]	0.210	0.210
		Dmed025	7.1×10^{-20}	0.001
		Dmed072	1.7×10^{-11}	0.059

CHR stands for chromosome.
Loci names are abbreviated, such as Dmed021 for Dmed^{UNICAMP}_ssr021.
[a]Loci present in the linkage map published by Laborda *et al.* (2012).
[b]BLAST results (E-values) of *D. mediopunctata* sequences matched to *D. melanogaster* homologous chromosome.
[c]BLAST results (smallest E-value) of *D. mediopunctata* sequences matched to *D. melanogaster* non-homologous chromosome.

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